Processing 2D NMR Data with MestReNova

1. Open the 2D Data File. On your PC or Mac you can rename the Bruker Experiment Number folder (1, 2, 3 …) to HSQC, COSY, HMBC, ROESY, etc. before dragging it into MestReNova. Drag this folder into the main window of MestReNova. For Varian data just drag the filename.fid folder into the MestReNova window.

2. Set Up Data Processing Method. These parameters control the peak shape and resolution in both dimensions, and different values are required depending on the type of 2D spectrum. Click on the drop-down menu **Processing** and select **Processing Template**. With the **f2** tab highlighted, Click on the button to the right and just below **Apodization**. Set the window function in F_2 as follows:

COSY: Sine Bell, 0.00 Deg
HSQC or TOCSY: Sine Bell, 45.00 Deg
HMBC, ROESY or NOESY: Sine Bell, 90.00 Deg (45.00 Deg if S/N is good)

Be sure to turn off (uncheck) any other Apodization settings besides Sine Bell. Click OK. Click on the next button down, next to **Spectrum Size**, and set to 2048. Click OK. Click on the **f1** tab. Make sure that the Truncate box is NOT checked. Click on the first button (…) under the **Apodization heading**, and set the F_1 window function as follows:

COSY: Sine Bell, 0.00 Deg
HSQC or TOCSY: Sine Bell, 45.00 Deg
HMBC (magnitude mode): Sine Square, 0.00 Deg
ROESY or NOESY: Sine Bell, 90.00 Deg

Be sure to turn off (uncheck) any other Apodization settings besides the one you want (Sine Bell or Sine Square). Click OK. Click on the next button (…) down, next to **Spectrum Size**, and set to 1024. Click OK. Click **OK** on the **Processing Template** window. It will take a few seconds as it re-processes the 2D data.

3. Choose Contour Display Setup. For consistency, always use red for positive intensities and blue for negative. Right-click on your 2D spectrum and select Properties (near the bottom). Click on **2D** (at the left) and select Red-Blue for **Palette**, Contour for **Plotting method**, 10 for **Number of Positive Contours**, 10 for **Number of Negative Contours**, and 1.100 for **Scaling** and 8.0 for **Line Width**. Click on **Set as Default** and then OK.

4. Open 1D Spectra for Traces. You will want to have a 1D spectrum corresponding to the F_2 dimension nucleus displayed along the top of the 2D rectangle, and another corresponding to the F_1 dimension nucleus displayed along the left side. MestReNova calls these 1D spectra **Traces**. For a heteronuclear inverse (HSQC or HMBC) spectrum, you will want a ^{1}H spectrum in the F_2 dimension and a ^{13}C spectrum for the F_1 dimension. For a homonuclear (COSY, ROESY, NOESY, TOCSY) spectrum you will want a ^{1}H spectrum in both dimensions.

Very Important: First, set the reference for the 1D spectra before using them as traces. Reference the ^{1}H spectrum using TMS or the residual solvent ($CHCl_3$, d_5-DMSO, etc.) peak, and reference the ^{13}C spectrum using the solvent peak ($CDCl_3$, d_6-DMSO, etc.). These reference values are posted in the NMR labs in tables of solvent and residual solvent shifts for all of the NMR solvents.

Right-click just above the top of the 2D display rectangle and select Show Traces with the right mouse button. This will put traces on the top (F_2 trace) and left side (F_1 trace) that are just projections of the 2D data, so the spectra will be broad and ugly. Right-click again on the top trace and select Setup with the right mouse button. Using the left mouse button now, select the ^{1}H spectrum in the list of Available 1D Spectra (proton spectra have an **H** at the left) and click on the top green arrow button (under Horizontal Trace). For a COSY spectrum, also click on the bottom green arrow button (under Vertical Trace). For an HSQC or HMBC spectrum, select the ^{13}C spectrum in the list of Available 1D Spectra (carbon spectra have an **C** at the left) and click on the bottom green arrow button (under Vertical Trace). When you have selected both traces (Horizontal and Vertical), click OK. Holding the cursor over the top (Horizontal) trace, you can use the mouse wheel to adjust the vertical scale of the 1D proton spectrum. Holding the cursor over the left side (Vertical) trace, you can use the mouse wheel to adjust the vertical scale of the F_1 trace.

5. 2D Phase Correction. For HSQC, this is phase-sensitive data, meaning that positive crosspeaks (red) are for CH and CH_3 groups and negative crosspeaks (blue) are for CH_2 groups. You will need to PHASE CORRECT the data to get these right. There is no need to phase correct 2D COSY or HMBC spectra. Be careful to make minimal changes to phase parameters because you can change the meaning (positive or negative) of the data by making big changes in the phase correction parameters. ROESY and NOESY are also phase sensitive (positive diagonal peaks and negative NOE crosspeaks) as is TOCSY (positive diagonal peaks and positive crosspeaks).

Phase errors appear in the HSQC spectrum as streaks coming out of a crosspeak. Errors in the horizontal (F_2) dimension are streaks to the left and right of the crosspeak: red on the left and blue on the right, or *vice-versa*. Errors in the vertical (F_1) dimension are streaks above and below the crosspeak: red above and blue below or *vice-versa*. To correct the errors, click on the drop-down menu at the right of the Phase Correction icon  and select Manual Correction. This brings up a phase correction box in the upper left corner. At the top, select **f2** to correct the horizontal dimension: 

. Below this is a blue rectangle with text instructions: "Click here and drag mouse up or down…". Ignore this for now and go to the slider at the bottom of the Phase Correction box. Move this left and right to position the vertical line on a crosspeak at the right side of the 2D spectrum. This is the "pivot peak". Now click and drag up and down GENTLY with the left mouse button on the blue rectangle with text instructions. You will see the phase errors in the horizontal dimension on the pivot peak. Move the mouse up and down to

minimize these phase error streaks around the pivot peak. Now look at another crosspeak, near the left side of the 2D spectrum, and use the right mouse button to click and drag up and down to correct the horizontal phase errors of this crosspeak.

To correct the vertical (F_1) phase errors, select **f1** at the top of the Phase Correction box: 

. Now use the slider to position the horizontal cursor on a prominent crosspeak near the top of the 2D spectrum. This will be the pivot peak. Click in the blue rectangle using the left mouse button to click and drag up and down to correct the phase errors (streaks above and below) for the pivot peak. Do the same with the right mouse button to minimize the phase errors for a prominent peak near the bottom of the 2D spectrum display. With both dimensions phase-corrected, click on the X in the upper right corner of the Phase Correction box.

6. Expand Region with Crosspeaks. Click on the magnifying glass (+) icon  or enter "e" at the keyboard. Click and drag from lower left to upper right of the region you want to expand. [To return to the full spectrum, Enter "f" or click on the full spectrum icon ]

7. Contour Plot. The default display is an intensity plot. To get a contour plot, right click on the 2D spectrum and select **2D Plotting Method** and **Contour Plot** in the drop-down menu. Adjust the contour threshold by clicking on the reduce intensity  button repeatedly until the least intense crosspeak disappears. Then click on the increase intensity button  until this weakest peak is again visible. Setting the threshold reduces the intensity of streaks and artifacts while making sure you don't miss any real crosspeaks.

8. Referencing the 2D Spectrum. You are wasting your time trying to reference the 2D spectrum if you have not first referenced the 1D spectra you have displayed as traces. If you have not done this, **go back** and reference these 1D spectra (step **4** above) and re-display the traces (Setup window for traces). The crosspeaks (and diagonal peaks in COSY) will not at first line up with the corresponding peaks in the 1D traces. To fix this, expand a crosspeak that you know should line up with a simple peak in the ^{1}H spectrum. In a COSY spectrum it should be an unoverlapped diagonal peak; in an HSQC or HMBC it should be a strong methyl crosspeak. Click on the Reference 

icon and hit the Shift key once to "free up" the cursor to move continuously without trying to "latch onto" a crosspeak. Line the vertical crosshair up precisely with a prominent peak in the top 1D proton spectrum, and note the exact value of the first number, the F_2 (proton) chemical shift.

HSQC or HMBC (F_2): Now put the vertical crosshair in the center of the crosspeak corresponding to the peak you used in the 1D proton (top) spectrum and click the left mouse button. In an HMBC be sure that you are not using a one-bond artifact peak; instead use the intense methyl proton crosspeaks. In the "Reference along f2" box, with

the blue "**f2**" button selected, enter the New Shift as the value you noted from the 1D proton spectrum. Click OK and make sure that the crosspeak now aligns perfectly with the 1D proton peak.

COSY (F_2 and F_1): If you are referencing a COSY spectrum, you can reference both dimensions at the same time by using a diagonal peak. Instead of aligning the vertical cursor with the diagonal peak, place the **center circle** (intersection of the vertical and horizontal cursors) in the center of the diagonal peak and click the left mouse button. With the blue "**f2**" button selected, enter the precise proton chemical shift from the 1D spectrum in the "New Shift" box. Then click on the red "**f1**" button and enter the same number in the "New Shift" box for F_1. Click OK and check to make sure that the 1D proton peak aligns with the diagonal peak in both (horizontal and vertical) dimensions.

HSQC and HMBC (F_1): After setting the reference in F_2, again click on the Reference  icon and hit the Shift key once to "free up" the cursor to move continuously. Now align the horizontal cursor precisely with a peak in the ^{13}C spectrum on the left side of the 2D display. Note the exact value of the second number (after the comma), which is the F_1 (carbon) chemical shift. Align the horizontal cursor with the center of the corresponding crosspeak (in an HMBC this can be a one-bond artifact pair for a methyl carbon) and click the left mouse button. Click on the red "**f1**" button and enter the same number in the "New Shift" box for F_1. Click OK and check to make sure that the 1D carbon peak now aligns with the crosspeak in the vertical dimension.

Especially with the ^{13}C (F_1) dimension of HSQC and HMBC, it's easy to reference the wrong crosspeak. Look at the whole spectrum and make sure the most upfield 1D peak corresponds to the most upfield crosspeak, and likewise with the most downfield peak. If all crosspeaks don't align with the 1D spectra, you need to start over with referencing.

9. Plotting. The best way to plot is to save the view as a pdf file. With the desired 2D display highlighted, click on **File** and **Export to PDF**, navigate to the correct folder and edit the filename, leaving the pdf extension unchanged. Click **OK** and then with **Current Page** selected click **OK** again. Then you can print from the pdf file to your printer or move the file elsewhere for printing.

10. Analysis. Click on the crosshair  icon or enter "c" at the keyboard. Position the crosshair on a crosspeak and "read" the 1D peak on the trace at the left and the other 1D peak on the trace at the top. These two 1D peaks are correlated by that crosspeak. For example, for an HSQC, the vertical line points to the ^{1}H peak in the top trace and the horizontal line points to the ^{13}C peak in the left side trace. You can also use the crosshair to measure distances, for example to extract J values from the fine structure of a COSY crosspeak.

11. 1D Slices. You can "slice" the 2D spectrum horizontally or vertically at any position to create a 1D spectrum or "slice". Usually this is done in the horizontal (F_2) dimension because the resolution is best in this dimension. For example, for a 2D HSQC spectrum a

horizontal (F_2) 1D slice would be a proton spectrum of just the protons directly attached to one carbon atom. The resolution will be worse than a 1D proton spectrum, so you won't see small splittings (J < 8 Hz). 2D slices are handled as "traces" in MestReNova. With the traces displayed on the top and left side of the 2D spectrum, click on the drop-down arrow at the right side of the **Show Traces** icon:  . This icon is located on the left side of the MestReNova frame, near the top. On the drop-down menu, click on **Select Traces Graphically**. This brings up the crosshair on the 2D spectrum. Position the crosshair on the crosspeak of interest and left-click. The horizontal slice at that position will be displayed above the 2D spectrum and the vertical slice will be displayed on the left side. If you're only interested in the horizontal slice, you can pay attention only to the horizontal line of the crosshair when you click.

You can also move the position of the slice up and down in the **Setup Traces** window. Right-click on the top trace (horizontal trace) above the 2D spectrum and select **Setup**. In the **Setup Traces** window, the green check mark is no longer highlighted and instead it says something like "Internal Trace #388 of 512 (31.628 ppm)" under the **Horizontal Trace** checkbox. To the right of this is a box with the number: 

. You can click on the up and down arrows to the right of the number to increase or decrease the number, moving the slice up or down in the 2D spectrum. The "Trace" shows this slice as you change the number. At any time in this process you can click on the **Extract Current Horizontal Trace as New Item** icon:  . This will take the 1D slice and add it as a 1D spectrum in your "Pages" at the left of the main display window. This spectrum can them be analyzed to extract coupling constants. To return to using the real 1D spectra (rather than slices) as the traces above and to the left of the 2D spectrum, highlight the ^{1}H spectrum in the **Available 1D Spectra:** display and click on the green check box under the **Horizontal Trace** heading. Do the same for the ^{13}C spectrum, clicking on the green check box under the **Vertical Trace** heading. Then click **OK**.